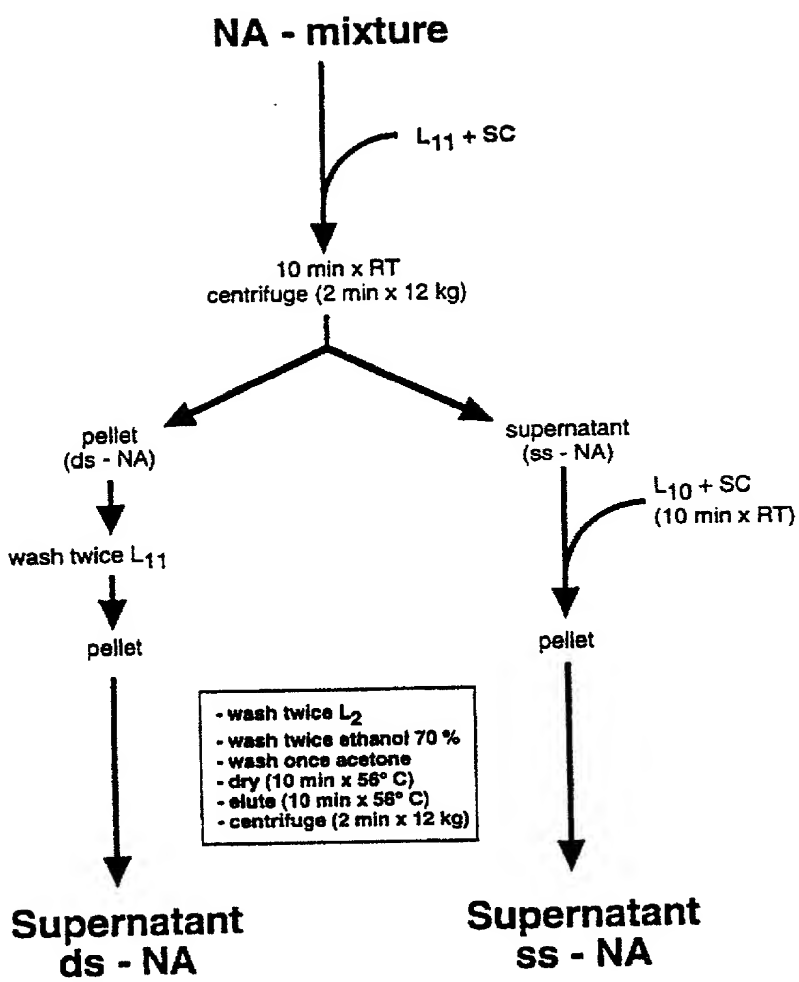

NA - mixture
L_{11} + SC
10 min x RT
centrifuge (2 min x 12 kg)
pellet
(ds - NA)
supernatant
(ss - NA)
L_{10} + SC
(10 min x RT)
wash twice L_{11}
pellet
pellet
- wash twice L_2
- wash twice ethanol 70 %
- wash once acetone
- dry (10 min x 56° C)
- elute (10 min x 56° C)
- centrifuge (2 min x 12 kg)
Supernatant
ds - NA
Supernatant
ss - NA

FIGURE 1

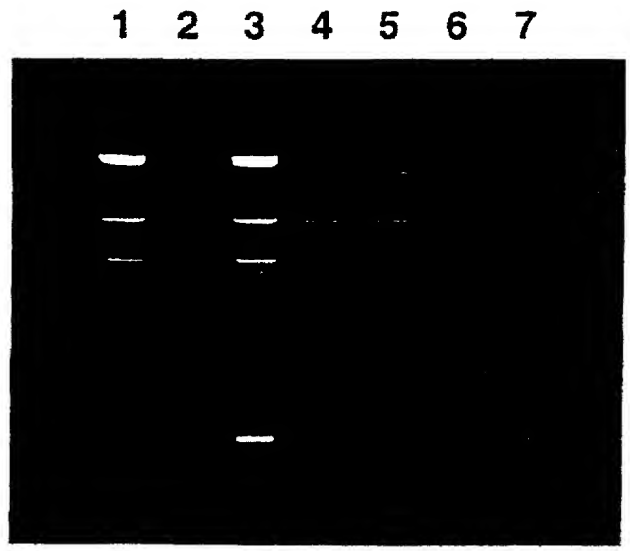


FIGURE 2

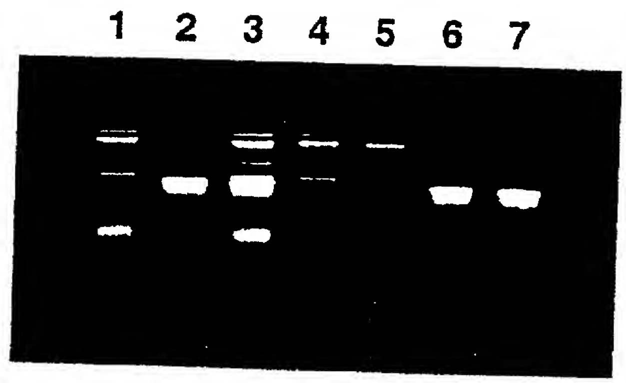


FIGURE 3

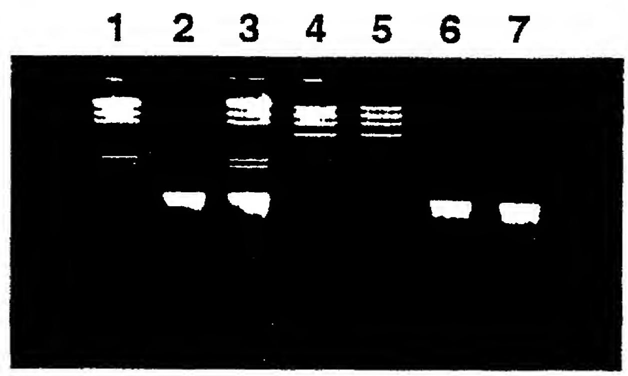


FIGURE 4

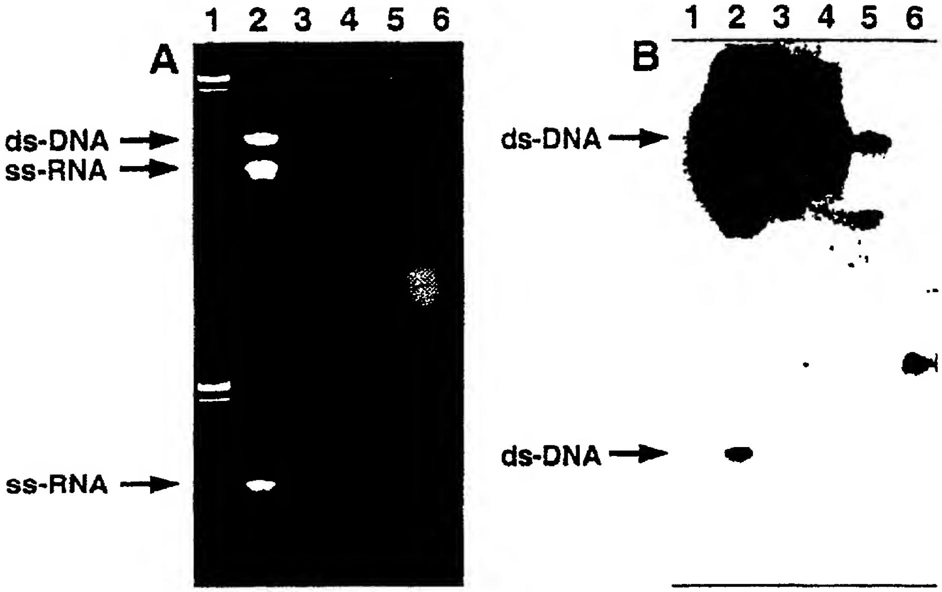


FIGURE 5

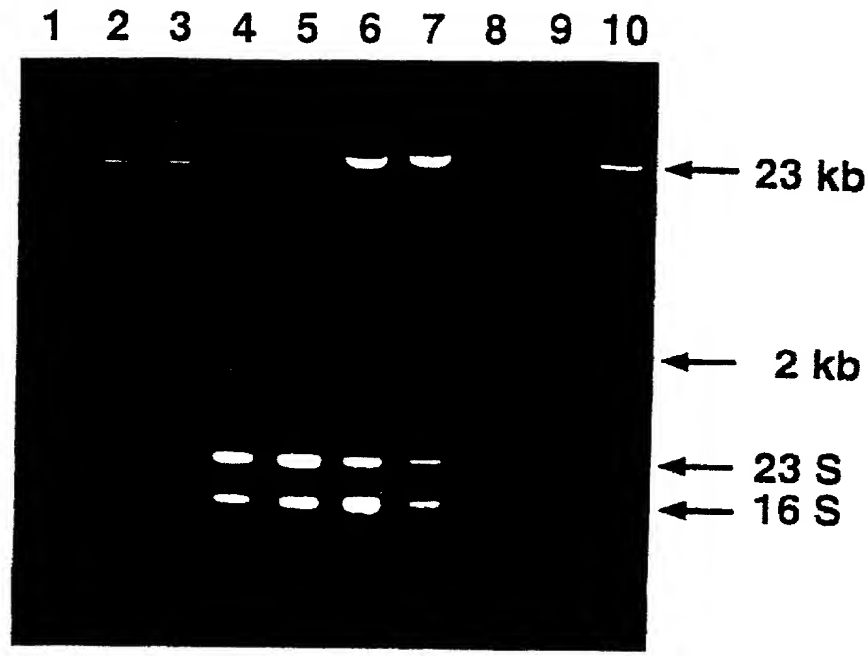
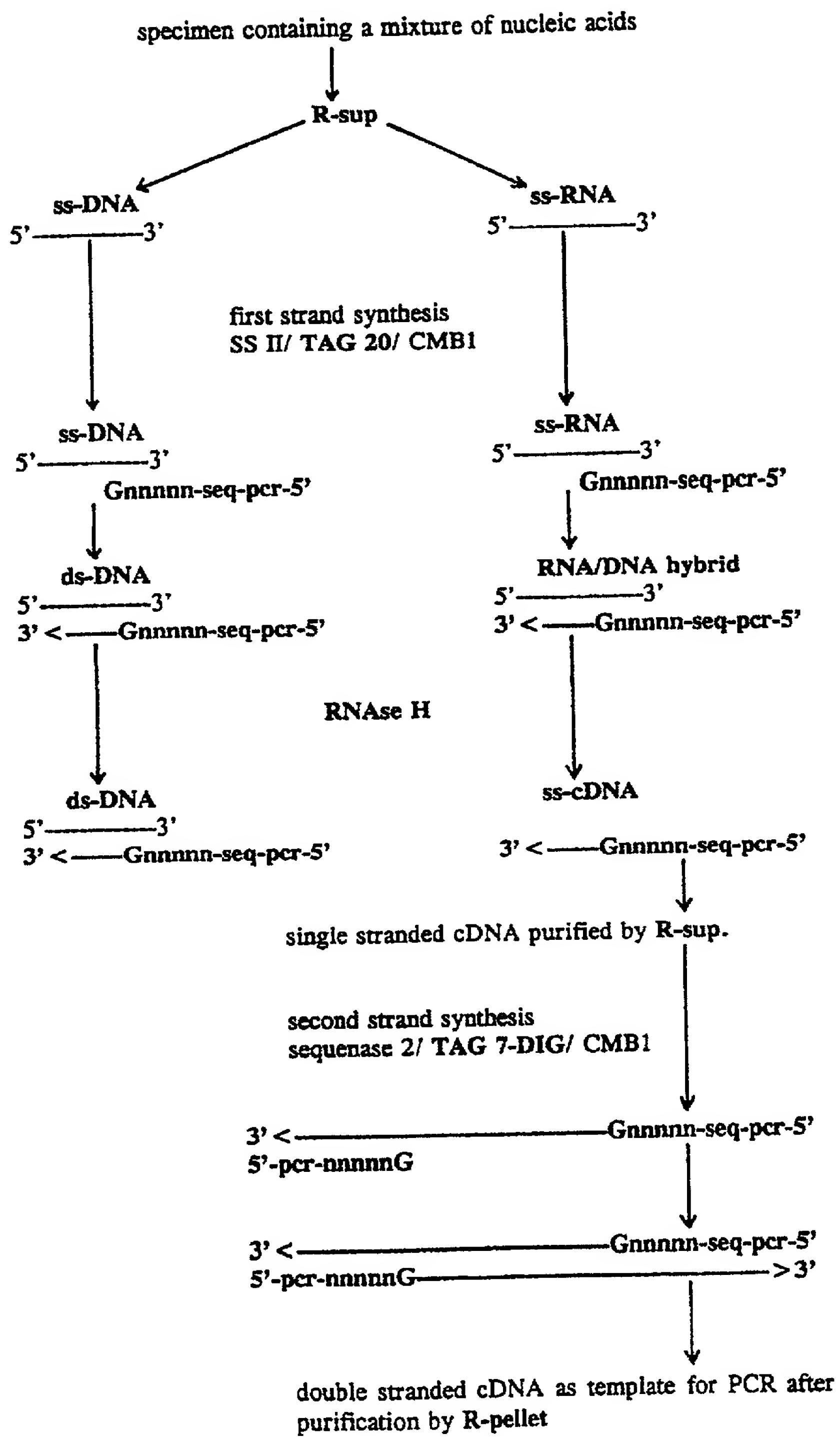


FIGURE 6

FIGURE 7


specimen containing a mixture of nucleic acids
R-sup
ss-DNA
5'——3'
ss-RNA
5'——3'
first strand synthesis
SS II/ TAG 20/ CMB1
ss-DNA
5'——3'
Gnnnnn-seq-pcr-5'
ss-RNA
5'——3'
Gnnnnn-seq-pcr-5'
ds-DNA
5'——3'
3' <——Gnnnnn-seq-pcr-5'
RNA/DNA hybrid
5'——3'
3' <——Gnnnnn-seq-pcr-5'
RNAse H
ds-DNA
5'——3'
3' <——Gnnnnn-seq-pcr-5'
ss-cDNA
3' <——Gnnnnn-seq-pcr-5'
single stranded cDNA purified by R-sup.
second strand synthesis
sequenase 2/ TAG 7-DIG/ CMB1
3' <——Gnnnnn-seq-pcr-5'
5'-pcr-nnnnnG
3' <——Gnnnnn-seq-pcr-5'
5'-pcr-nnnnnG——> 3'
double stranded cDNA as template for PCR after purification by R-pellet

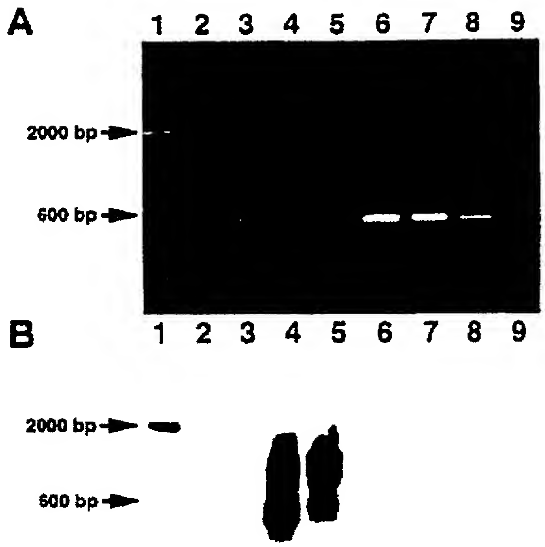


FIGURE 8

line 31, delete "wanted" and replace with -- desired --.

Page 2, line 14, after "DNA", delete ", an" and replace with -- . An --;

between lines 20 and 21, insert -- Summary of the Invention --;

line 21, delete "therefor" and replace with -- , therefore, --

between lines 30 and 31, insert --

Brief Description of the Drawings

Figure 1 is an outline of protocol R. Recovery of ds-NA takes place from the initial pellet (R-pellet), recovery of ss-NA takes place from initial supernatant (R-sup). L11, L10, L6 and L2 are GuSCN-based buffers, SC is silica particle suspension.

Figure 2 shows the separation of ds-DNA and ss-DNA. NA was purified (in duplicate) by protocol R from a mixture of ds-DNA (phage lambda, HindIII digest, 1μg) and ss-DNA (phage M13 DNA, 500ng). Final elutions were in 50μl TE and 25μl were electrophoresed through a 1% agarose gel (containing ethidium bromide), which was subsequently photographed under UV-illumination. Lane 1, 100% recovery marker for ds-DNA fragments; lane 2, 100% recovery marker ss-DNA; lane 3, 100% recovery marker mixture ds-DNA/ss-DNA. Lanes 4 and 5, output protocol R-pellet; lanes 6 and 7, output protocol R-sup.

Figure 3 shows the separation of ds-RNA and ss-RNA. NA was purified (in duplicate) by protocol R from a mixture of ds-RNA (Rotavirus ds-RNA) and ss-RNA (phage MS2 RNA, 800ng). Final elutions were in 50μl TE and 25μl were electrophoresed through a 1% agarose gel (containing ethidium bromide) which was subsequently photographed under UV-illumination. Lane 1, 100% recovery marker for ds-RNA fragments; lane 2, 100% recovery

marker ss-RNA; lane 3, 100% recovery marker ds-RNA/ss-RNA mixture. Lanes 4 and 5, output protocol R-pellet; lanes 6 and 7, output protocol R-sup.

Figure 4 shows the separation of ds-DNA and ss-RNA. NA was purified (in duplicate) by protocol R from a mixture of ds-DNA (750ng phage lambda digested with hindIII) and ss-RNA (phage MS2 RNA, 800ng). Final elutions were in 50μl TE and 25μl were electrophoresed through a 1% agarose gel (containing ethidium bromide) which was subsequently photographed under UV-illumination. Lane 1, 100% recovery marker for ds-DNA; lane 2, 100% recovery marker for ss-RNA; lane 3, 100% recovery marker for ds-DNA/ss-RNA mixture. Lanes 4 and 5, output protocol R-pellet; lanes 6 and 7, output protocol R-sup.

Figures 5A and 5B show the separation of ds-DNA and ss-RNA. NA was purified by protocol R-sup from a mixture of ds-DNA (1000ng linearized pHC624, 2kb) and ss-RNA (phage MS2 RNA, 800ng). Final elution was in 50μl TE and 25μl or tenfold serial dilutions of the ss-NA fraction were electrophoresed through a 1% agarose gel (containing ethidium bromide) which was subsequently photographed under UV-illumination. Figure 5A: upper row, lane 1, HindIII digested phage lambda DNA; lane 2, 100% recovery marker for ds-DNA and ss-RNA and serial tenfold dilutions thereof (lanes 3 - 6). Bottom row, output of protocol R-sup (lane 2) and tenfold serial dilutions (lanes 3 - 6). Figure 5B: ds-DNA was subsequently transferred to a nitrocellulose filter and hybridized with a ^{32}P-labelled probe homologous to input ds-DNA. ds-DNA and ss-RNA are indicated.

Figure 6 shows the separation of genomic DNA from ss-RNA and how to deal with trapping of ss-RNA. *E. coli* were directly used as input material for duplicate extractions by protocol R (lanes 6 and 7, R-pellet: lanes 8 and 9). Alternatively, total NA was first purified by protocol Y using diatoms as NA-carrier (which

causes shearing of genomic DNA). The purified nucleic acids were subsequently used as input for protocol R (lanes 2 and 3, R-pellet; lanes 4 and 5, R-sup). Final elutions were in 50μl TE and 25 μl were electrophoresed through a 1% agarose gel (containing ethidium bromide) which was subsequently photographed under UV-illumination. Marker lanes 1 and 10 (500ng phage lambda DNA, HindIII digested). 23S and 16S rRNA, and ds-DNA molecular weight markers (23kb and 2.0kb) are indicated.

Figure 7 is an outline of the procedure.

Figures 8A and 8B show how single-stranded nucleic acid was purified from samples containing HIV-1 RNA and TE (negative control) by protocol R-sup. and subsequently amplified with the non-selective RT-PCR. Panel A: lane 1, 100 bp DNA ladder; lanes 2 and 3 negative extraction controls; lanes 4 and 5 non-selectively amplified HIV-1 RNA; lanes 6, 7, 8 and 9 600, 60, 6 and 0 molecules resp. of pHCrec (positive PCR control). Panel B: Southern blot hybridization with ^{32}P-labelled HIV-1 probes (containing the GAG, POL and ENV genes of HIV-1) of the samples shown in panel A. After overnight hybridization at 65°C in 6 x SSC, 0.1% SDS, 10% Dextran Sulphate and 50μg/ml salmon sperm DNA, the filter was subsequently washed under high stringency conditions with 0.1 SSC/0.1% SDS at 65°C, and autoradiographed on X-ray film for two hrs. at -70°C. This experiment showed that most of the bands visible on the ethidium bromide stained agarose gel originated from the HIV-1 genome.

Detailed Description of the Invention --; and

line 37, delete "that EDTA is applied" and replace with -- of EDTA --.

Page 3, line 12, delete "basepairs" and replace with -- base pairs --;

line 15, delete "a.o." and replace with -- , among others, --; and

line 22, delete "effect" and replace with -- affect --.

Page 4, line 28, delete "when" and replace with -- that at which --.

Page 6, line 7, delete "ethidiumbromide" and replace with -- ethidium bromide --; and

line 10, after "strands,", insert -- and --.

Page 7, line 10, delete "Guanidiumthiocyanate" and replace with -- Guanidinium thiocyanate --.

Page 8, line 18, delete "silica-pellet" and replace with -- silica pellet --.

Page 9, line 3, delete "ethidiumbromide" and replace with -- ethidium bromide --;

line 17, delete "lysisbuffers" and replace with -- lysis buffers --; and

line 36, delete "silica-" and replace with -- silica --.

Page 10, line 35, after "Again", delete "are" and after "fractions", insert -- are --.

Page 11, line 3, delete "ethidiumbromide" and replace with -- ethidium bromide --; and

line 11, delete "0,1%" and replace with -- 0.1% --.

Page 15, line 5, delete "microliter" and replace with -- microliters --; and

line 19, delete "Twently microliter was taken of" and replace with -- Twenty microliters was taken off --.

Page 16, lines 4, delete "Twentyfive microliter" and replace with -- Twenty-five microliters --;

line 6, delete "ethidiumbromide" and replace with -- ethidium bromide --; and

line 20, delete "ethidiumbromide" and replace with -- ethidium bromide --.

Page 17, line 3, delete "HIV-origin" and replace with -- HIV origin --.

Page 21, delete "CLAIMS" and replace with -- We claim: --.

IN THE CLAIMS:

Please cancel claims 1 - 15 without prejudice or disclaimer of the subject matter thereof.

Please add the following new claims 16 - 37:

-- 16. A method for separating single stranded nucleic acid from double stranded nucleic acid, comprising the steps of:

contacting a mixture of both with a first liquid comprising a chaotropic agent and a nucleic acid binding solid phase, wherein the first liquid has a composition such that the double stranded nucleic acid binds to the solid phase;

separating the solid phase from a supernatant containing the single stranded nucleic acid; and

treating the supernatant with a second liquid comprising a chaotropic agent and a second nucleic acid binding solid phase, wherein the second liquid has a composition such that the resulting mixture of supernatant and second liquid allows for binding of the single stranded

nucleic acid material to the second solid phase, whereby the single stranded nucleic acid is isolated. --

-- 17. The method according to claim 16, wherein the first liquid comprises a chaotropic agent in concentration between about 1-10M, and a chelating agent, and has a pH between about 2 and 10. --

-- 18. The method according to claim 17, wherein the chelating agent is EDTA, which is present in a concentration between about 10 mM and 1M. --

-- 19. The method according to claim 18, wherein the first liquid comprises at least about 100mM EDTA and guanidinium salt as a chaotropic agent. --

-- 20. The method according to claim 16, wherein the chaotropic agent is guanidinium thiocyanate. --

-- 21. A method according to claim 20, whereby the first liquid has the constitution of a buffer prepared by dissolving about 120g guanidinium thiocyanate in about 100ml 0.2M EDTA (pH=8). --

-- 22. The method according to claim 16, wherein the second liquid comprises a chaotropic agent, a chelating agent and divalent positive ions. --

-- 23. The method according to claim 22, wherein the concentration of the divalent positive ions is the same as the concentration of the chelating agent. --

-- 24. The method according to claim 22, wherein the chelating agent is EDTA and the ions are Mg^{2+} ions. --

-- 25. The method according to claim 22, wherein the chaotropic agent is a guanidinium salt. --

-- 26. The method according to claim 25, wherein the guanidinium salt is guanidinium isothiocyanate. --

-- 27. The method according to claim 26, wherein the second liquid has the constitution of a buffer prepared by dissolving about 120g guanidinium isothiocyanate in about 100ml 0.35M TRIS HCL (pH 6.4) and adding about 22ml 0.2 M EDTA (pH 8.0) and about 9.1g Triton X-100, homogenizing the solution and adding $MgCl_2$ to a final concentration of about 0.25M. --

-- 28. The method according to claim 16, wherein the solid phase is silicium based. --

-- 29. The method according to claim 28, wherein the solid phase is silica. --

-- 30. The method according to claim 29, wherein the silica is in the form of particles having a size between about 0.05 and about 500 micrometers. --

-- 31. The method according to claim 16, wherein the solid phase is separated from the supernatant by centrifugation. --

-- 32. A method for preparing double stranded cDNA from single stranded RNA, comprising the steps of:

contacting the single stranded nucleic acid with a first primer, said first primer comprising a random hybridization sequence and amplification motif, and an enzyme having RNA dependent DNA polymerase activity to obtain first strand cDNA synthesis by creating a DNA/RNA hybrid;

rendering the obtained RNA/DNA hybrid single stranded by contacting the hybrid with an enzyme having RNase H activity; and

contacting the single stranded cDNA with an enzyme having DNA dependent DNA polymerase activity and a second primer comprising a random hybridization sequence and amplification motif, whereby double stranded cDNA is obtained. --

-- 33. The method according to claim 32, wherein the amplification motif in the first and second primers is the same. --

-- 34. The method according to claim 32, wherein at least one of the primers comprises a direct sequencing motif. --

-- 35. The method according to claim 32, wherein at least one of the primers comprises a label. --

-- 36. The method according to claim 32, wherein the obtained cDNA is further amplified in a nucleic acid amplification reaction using at least one primer that specifically anneals to the amplification motif. --

-- 37. The method according to claim 32 wherein the single stranded RNA comprises mRNA. --

REMARKS

Claims 1 - 15 are canceled and claims 16 - 37 are added hereby. New claims 16 - 37 have been added to better define Applicants' invention and/or for purposes of clarification unrelated to patentability concerns.

It is believed that claims 16 - 37 recite a patentable improvement in the art. Favorable action is solicited.

Applicants respectfully request that the sequence listing in computer readable form (CRF) filed in the parent application

(USSN 09/125,241, filed August 13, 1998) on January 18, 2000, in response to the Notice to Comply, be used to prepare a file for the present application. The paper copy of the sequence listing filed with the present application is identical to the sequence listing in that CRF.

In the event any fees are required with this paper, please charge our Deposit Account No. 02-2334.

Respectfully submitted,

Michael G. Sullivan
Attorney for Applicants
Registration No. 35,377

Attorney Docket No. T/96235 US/C1

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